VIRUS RETENTION BY SELECTED INDIAN SOILS

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CERTIFICATE

Certified that the work presented in this thesis entitled 'Virus Retention by Selected Indian Soils', by Sri K.V. Abdulla Koya has been carried out under my supervision and it has not been submitted elsewhere for a degree.

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VIRUS RETENTION BY SELECTED INDIAN SOILS

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A review of the literature revealed that studies on movement or retention of viruses by soils were very few and no data were available regarding the virus sorption potential of Indian soils. The objective of the present study was to evaluate the potential of four selected Indian soils, viz., Beach sand (Malabar, Kerala), Black Cotton soil (Madhya Pradesh), Kanpur silt (Uttar Pradesh) and Lateritic soil (Kerala) in removing viruses from percolating waters. Batch sorption tests at different pH (6.0, 7.0, 7.8 and 8.4) and column studies using a natural groundwater supply were conducted in the laboratory using bacteriophage MS2 against Escherichia coli as the model virus.

Lateritic soil, Black Cotton soil and Kanpur silt were found quite effective in removing the model virus from water both in the batch sorption tests as well as in the column studies. The sorptive capacity of soil was found to be dependent on its clay content and the pH of the system. No apparent correlation was observed between the sorption of

viruses and cation exchange capacities of soil. From the equilibrium sorption tests, the sorptive capacity of the soils were as follows: Beach sand - 1.6×10^6 PFU/g; Black Cotton soil - 7.8×10^6 PFU/g; Kanpur silt - 2.16×10^6 PFU/g; and Lateritic soil - 4.2×10^7 PFU/g. From the column studies it was observed that a 7.5 cm depth of Lateritic soil was much more efficient than Black Cotton soil and Kanpur silt of 15 cm depth in removing the model viruses. Breakthrough of the model virus through sand was immediate apparently due to rapid drainage of percolant through the sand columns and its poor sorption potential.

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I. INTRODUCTION

'Clear and safe water' is not a recent concept in environmental engineering. Transmission of diseases, particularly those associated with human enteric viruses through the water route has become a source of major concern among the environmental engineers working in the laboratories as well as in the field. Within the last three decades there have been numerous epidemics including several outbreaks of viral diseases which have been attributed to water-borne viruses. Transmission of viral diseases through the water route has been established (Clarke and Chang, 1959). Mosley (1966) presented an extensive review of water-borne outbreaks of enteric viral diseases including the 1955-56 infectious hepatitis epidemic of New Delhi. Seventy one percent of the 53 outbreaks of infectious hepatitis which occurred in the United States during the period 1946-1970 were attributed to contaminated water supplies (Craun and Macabe, 1973). Also, a great deal of information is available regarding the presence of viruses in recreational waters (Merrell and Ward, 1968 and Kelly and Sanderson, 1961).

Viruses are excreted in large quantities with the feces of infected people, particularly with those of the infected children who have high carrier rates for enteroviruses

More than 100 types of viruses are known to be excreted by man and the presence of more than 70 of them have been demonstrated in wastewater (Clarke and Chang, 1959), which may ultimately find their way into surface or groundwater. Several outbreaks of viral diseases were traced to polluted well waters (Vogt, 1961). Recently poliovirus was recovered from the water of a contaminated well used by a large restaurant in Michigan (Mack, 1973). Peczenick, et al. (1956) traced an epidemic of infectious hepatitis to contaminated water used by an Austrian Hotel. The source of water supply to this hotel was contaminated by the effluent from the septic tank of an adjacent hotel. In most of these cases viruses appeared to have travelled through the cracks and fissures of soils into the groundwater.

Though there have been well documented cases of waterborne viral diseases attributed to contaminated groundwater supplies, available laboratory as well as the field data concerning the transmission and survival of viruses in soil-water systems are quite limited. Relatively, a very few studies were undertaken on virus movement in groundwater in the United States (Eliassen and Kruger, 1964, Eliassen, et al., 1965, Drewry and Eliassen, 1968, Eliassen, et al., 1967, and Drewry, 1969). The objective of this study was to investigate the relative capacities of a few selected Indian soils in

removal or inactivation of viruses from water. It was thought that such a study would enable the environmental engineer to assess the contamination of groundwater supplies with regard to water-borne viruses when virus-laden effluents are discharged to land or disposed through engineered soil percolation systems.

II. LITERATURE REVIEW

A. Viruses

(1) Physical and chemical properties

Viruses are proteinaceous particles of submicroscopic size, having a protein shell or capsid and containing large amounts of nucleic acid, DNA or RNA, or both, within these shells. All viruses are obligate intracellular parasites which can multiply only within living host cells. The ability to maintain genetic continuity with the possibility for mutations is the only basis for considering viruses to be alive (Goodheart, 1969). Viruses which are known to infect man, animal and plants exist in a variety of size and shapes. Viruses of the same type have the same shape and size and this striking property of uniformity has been established by electron microscopic study (Luria and Darnell, 1967). Viruses fall into one of three broad structural groups, viz., roughly spherical, elongated rod-shaped, or tadpole shaped with head and tail. Some viruses are known to contain an enzyme which can bore holes in host cell walls (Burnet and Stanley, 1970). Viral protein is not necessary for viral replication; it aids attachment and penetration of the virus into its host cell and serves as a protective shell. Viral nucleic acid maintains the genetic continuity

of viral replication. Structural differences in viral proteins make viruses host specific (Stent, 1963).

The bacterial and animal viruses have many similar physical, chemical and biological properties (Adams, 1959). Animal viruses range in size from about 10 to 450 nm whereas the bacterial viruses range between 20 and 95 nm. Table 1 shows the physical and chemical properties of some human enteroviruses and bacteriophages. Viruses are sensitive to environmental conditions like pH, temperature, UV light, etc.

(2) Water-borne enteric viruses and diseases associated with them

Enteric viruses significant in water supply practice are the enteroviruses (poliovirus, cosackievirus and echovirus), infectious hepatitis virus(es), the adenovirus and the reovirus. Table 2 lists the main enteric viruses and the diseases with which they have been closely associated.

Infectious hepatitis is the only viral disease which has been epidemiologically established to be water-borne (Mosley, 1966). Twenty one cases of infectious hepatitis have been well documented and they have been attributed to the contaminated water supplies (Berg, 1964). Though evidence is lacking to label poliomyelitis virus as water-borne, cases of poliomyelitis occur concurrently with infectious hepatitis in India (Balasubramaniam, 1967). Walter et al. (1972) recovered poliovirus from a contaminated well

TABLE 1

Comparison of Physical and Chemical Properties of Some Human Enteroviruses and Bacteriophages (After Fraenkel-Conrat, 1968)

Virus	Size nm	$S_{20,w}$	CsCl density g/ml	Molecular weight x 10^{-6}	Mol.wt. of nucleic acid x 10^{-6}	Nucleic acid percent	Composition of nucleotides present			
							Adenine	Guanine	Uracil	Cytosine
Polio	27-30	154-160	1.58	6.8-6.9	2.2	29	29	29	25	22
Coxsac-kie	27-30	153				30				
MS2 phage	25-26	79-81		3.6-3.7	1.05	31	23	26	26	25
R17 phage	23-26	79-80	1.46	3.74	1.1-1.2	31	23	26	26	25
T2 phage	65x95 20x95			300	120	44	32.4	18.3	32.4(T)	0 (5HMC 17)
T4 phage	65x95 20x95	700 and 1000		200	120	60	32.3	18.1	33.3	0 (5HMC 16.

TABLE 2

Enteric Viruses and Diseases with which They have been Closely Associated (After Berg, 1966)

Virus[φ]	Disease								
	Paralytic Poliomyelitis	Aseptic Meningitis	Pleurodynia	Herpangina	Respiratory Illnesses	Enteritis	Rash Diseases	Acute Infantile Myocarditis	Jaundice
Polioviruses	X	X	-	-	-	-	-	-	-
Coxsackieviruses group A	-	X	-	X	-	-	-	-	-
Coxsackieviruses group B	-	X	X	-	-	-	-	X	-
Echoviruses	-	X	-	-	X	X	X	-	-
Adenoviruses	-	-	-	-	X	-	-	-	-
Reoviruses	-	-	-	-	X	X	-	-	-
Infectious hepatitis virus(es)	-	-	-	-	-	-	-	-	X

[φ] Only certain strains within the designated groups have been proven to be responsible for the designated diseases.

used by a large Michigan restaurant, patrons of which became suddenly ill within 30 hours of a meal. Many public health workers believe that enteroviruses are responsible for many of the episodes of gastroenteritis; however, adequate documentation is lacking. The transmission of causative agents of gastroenteritis by the water route has been reported by Kelly and Sanderson (1959). Recently, outbreaks of gastroenteritis have been attributed to water-borne viral agents (Craun and Macabe, 1973). Table 3 shows the outbreaks of viral disease data of United States during the period of 1946-1970. The majority of the infectious hepatitis outbreaks in public system occurred as the result of contamination of the distribution system; for private system, contamination of untreated ground-water supplies was the important factor.

Plotkin and Katz (1967) reported that one cell culture infective dose is sufficient to infect a man. This indicates that if viruses are isolated from water that is consumed by man, there is sufficient virus in that water to infect a proportion of those that consume the water. However, the epidemiological data available so far are insufficient to give a measure of what this proportion is and it is interesting to note that in Paris, France, viruses were readily isolated from tap water (Coin, et al., 1965) with no evidence of corresponding outbreaks of viral diseases.

TABLE 3

Outbreaks of Water-Borne Viral Diseases in U.S.A. During the Period 1946-1970 (After Craun and Macabe, 1973)

Disease	Private system		Public system		Total	
	Outbreaks	Cases	Outbreaks	Cases	Outbreaks	Cases
Gastroenteritis	121	8970	57	36285	178	45255
Infectious hepatitis	36	1094	17	739	53	1833
Poliomyelitis	-	-	1	16	1	16

(3) Survival of viruses in water and their densities

A number of investigators have isolated viruses from contaminated water (Kelly and Sanderson, 1959). Density of enteric viruses in surface waters varies with the predominance of virus species and the physico-chemical quality of the water. The most resistant viruses can survive upto 200 days in river water (Cookson, 1974). Viruses are generally stable in the pH range of natural waters. Survival times of enteric viruses also depend on salt concentration, particularly polyvalent cations (Chang, et al., 1958). Studies on survival of viruses in water show that both polio and coxsackie viruses remained active in distilled, tap or river water for many months (Gilcreas and Kelly, 1955). Viruses survive longer in relatively unpolluted or grossly polluted water than in mildly polluted water (Clarke, et al., 1962). It is generally believed that viruses may well survive longer in cooler groundwater than in warmer surface water.

Regarding the density of human enteric viruses in domestic wastewater, it was observed that their concentration varied seasonally, reaching a peak during the late summer and early fall (Gelfand, 1961). The average enteric virus density in raw domestic wastewater was estimated by Clarke et al. (1964) at 700 virus units per 100 ml. From the data available in the literature it appears that the average number in domestic

wastewater is probably about 600 $TCID_{50}$ per 100 ml during the warm months and 5 $TCID_{50}$ per 100 ml during cold months (Grabow, 1968). However, density as high as 10,000 $TCID_{50}$ per 100 ml has also been reported (Lund et al., 1969). A secondary effluent might be expected to contain 7 to 70 virus units per liter if no reduction is taken for primary treatment, 90 percent for activated sludge and 90 to 99 percent for chlorination, and if the initial enteric virus density is about 700 virus units per 100 ml (Sproul et al., 1967). Taking an initial density of 600 $TCID_{50}$ per 100 ml, 6 to 60 $TCID_{50}$ per liter can be expected in secondary effluents. This has also been confirmed by Lund, et al., (1969) from actual plant data.

Regarding surface waters the safe conclusion is that viruses are present in such waters and that failure to isolate them results presumably from their low concentrations, and the limitation of sampling and concentration procedures employed (Committee Report, 1970). Clarke, et al. (1964) estimated an enteric virus density of 10 PFU/1 in polluted surface waters. The reported values are 0.5 to 10 PFU/1 in the River Thames (Hill, et al., 1971), 13 PFU/1 in the Jordan River (Committee Report, 1970) and 45 to 286 PFU/gallon in a Texas stream (Grinstein, 1970).

B. Removal or inactivation of viruses by treatment processes

(1) Water treatment processes

Because of a lack of a simple reliable technique to quantitatively detect a small number of viruses in large volumes of water, a thorough in-plant evaluation of the reliability of modern water treatment techniques to remove or inactivate viruses has become a difficult task. A recent study of Clarke, et al. (1974) in ten water treatment plants in U.S.A. using the most advanced virus sampling and concentration techniques fail to draw any definite conclusions on the potential problem of water-borne viral diseases. Most of the data on virus removal by water treatment processes reported in the literature were obtained in laboratory systems using model viruses.

Carlson, et al. (1942) and Kempf, et al. (1942) studied the effect of alum coagulation in removal of poliovirus from water and observed that low alum dosages were not effective in rendering the water free from viruses. Chang, et al. (1958) reported that the removal of viruses during coagulation was reduced in the presence of phosphate buffer. Chaudhuri and Englebrecht (1970) and David and Drewry (1974) observed that alum coagulation was capable of removing 90 - 99 percent of the added virus from water; however, the removed viruses were not inactivated and remained active in the floc.

Virus removal was attributed to the formation of a virus-coagulant complex formation.

In the last few years, a very few studies were undertaken on virus removal by filtration (Sproul, 1971). A few observations were made regarding virus removal by filtration using sand, coal and garden soil as virus retention media (Carlson, et al., 1942, Kempf, et al., 1942, Gilcreas, 1955, Robeck, et al., 1962, and Oza, 1974). Gilcreas and Kelly (1955) observed incomplete removal of viruses on filtration through garden soil. Table 4 shows available information on virus removal by filtration. Poor removals were observed with sand filtration in general except when prior coagulation-flocculation was employed.

The resistance of virus to disinfection varies with the chemical agents employed and also with the type of viruses. It has been experimentally demonstrated that a free chlorine residual of 0.2 - 0.3 mg/l at 20°C and pH value not higher than 8.0 - 8.5 will destroy most of the tested viruses in a contact time of 30 min (Committee Report, 1970). A 99.9 percent inactivation of viruses with 0.7 mg/l ozone was reported by Perlman (1969). Diaper (1968) reported ozone residuals of 0.1 - 0.2 mg/l with a contact time of 5 min to be adequate for inactivation of all enteric viruses.

TABLE 4

Virus Removal by Filtration (After Amirhor, 1974)

Investigators and System	Flow Rate gpm/sq. ft.	Virus Used	Virus Removed Percent or as Noted
Carlson et al. (1942)			
Rapid Sand Filtration	2	Poliomyelitis (Pathogen for Mice)	Poor
Impregnated Filter	1.3		Good
Kempf et al. (1942)			
Flocculation and Rapid Sand Filtration	2	Poliomyelitis (Strain DG)	Poor
Gilcreas and Kelly (1955)			
Percolation Through 3 ft Soil		Coxsackie	50
		T4	20
Sand Filtration	0.2	Coxsackie and T4	99
	2	Coxsackie	10
Flocculation and Rapid Sand Filtration		T4	40
		Coxsackie	90
Impregnated Rapid Sand Filter		T4	99
		Coxsackie	90
Robeck et al. (1962)			
Sand Filtration - Slow Rate	0.035	Poliovirus 1	22 - 96

(2) Wastewater treatment processes

Clarke, et al. (1962) studied the reduction of viruses during storage with respect to time and temperature. For 99.9 percent reduction of certain viruses a storage period as long as 130 days was required. The reduction or inactivation of virus was dependent on temperature, time of storage, virus species and degree of pollution. Primary clarification results in minor removal of enteroviruses (Mack, 1962).

The activated sludge process has been shown to effect significant and consistent removal of viruses (Clarke, et al., 1961). They observed that 60 - 75 percent removals occurred when sewage containing virus was aerated for 6 - 8 hrs. Kelly and Sanderson (1959) reported only 40 percent removal of viruses by trickling filter. A review of the published data indicates that virus removal by trickling filter is poor and inconsistent whereas the activated sludge process is capable of removals on the order of 90 percent or more (Committee Report, 1970).

Waste stabilization ponds are effective in reducing the level of virus in raw wastewater and secondary effluents (Chaudhuri, 1973). Over 90 percent removals were reported in waste stabilization ponds (Arceivala, et al., 1971). The various parameters that influence the inactivation or removal of viruses in stabilization pond are a) presence of bacteria

and algae, b) chemical and organic contents of the pond water, c) detention time and temperature, d) high oxygen content, and e) sunlight (Chaudhuri, 1973).

C. Movement of viruses through soil

A survey of the literature indicates that very few investigations on virus movement through soil have been undertaken so far. The oldest study in this area was undertaken by Gilcreas and Kelly (1955) who recovered 50 percent coxsackievirus and 75 percent bacteriophage from percolant that passed through a 36 in. column of soil. However, many cases of infectious hepatitis caused by contaminated groundwater supplies were reported (Eliassen and Cumnig, 1949, Peczenick, et al., 1956, Clarke and Chang, 1959, and Weibel, et al. ,1964). In majority of these cases the groundwater supplies were contaminated by effluents from cesspools or septic tanks.

From their study on virus removal (T_1, T_2 and f_2 bacteriophages) using nine different soils from California and Arkanas, Drewry and Eliassen (1968) showed that all soils were effective in removing over 99 percent of the viruses. They concluded that movement of virus through continuous strata of common soils under saturated flow conditions should pose no health hazard to water supply wells. Later, Drewry (1969) observed 99 percent virus adsorption on three of the four soils studied using f_2 bacteriophages. He used effluents

from cesspools and septic tank system for preparing the feed solution and observed that the third soil exhibited decreased adsorption because of high content of sand which render less surface area per unit weight than the other three soils.

The study of Tanimoto, et al. (1968) on virus (T_4 bacteriophage) removal by the three Hawaiian soils showed that except one soil, i.e., gravel sized cindery material the other two soils were effective in virus removal. More than 95 and 99 percent of virus was removed by 1.5 and 6 in. soil columns of Wahaiva and Lahaina soils, respectively. Young and Burbank (1973) observed that only 35 percent of virus was removed or retained by 15 in. column of Tantalus soil which was characterised by rapid drainage. They also observed that even 6 in. columns were unable to effect 100 percent retention of poliovirus with an initial feed concentration of 1.5×10^5 PFU/ml. One hundred percent of T4 phage was observed at a concentration of 1.5×10^6 PFU/ml in a 2.5 in. soil column. The removal of poliovirus with sand was even less.

Soils vary in their capacities for removing viruses. Filmer and Corey (1966) observed that soils high in clay content were most effective in removing virus-sized albumin particles from water. Major field tests concerning the movement of viruses in groundwater were conducted by Merrell and Ward (1968) at the Santee water reclamation project at

Santee, California. The removal of virus in the passage of stabilization pond effluent through the percolation zone was very efficient. Robeck, et al. (1962) studied the removal of poliovirus in sand under natural groundwater flow rates. They observed that the sand beds removed 99 percent of the viruses for 98 days at natural groundwater flow rate of 1/128 gpm/sq.ft. Lefler and Kott (1974) studied the retention and survival of poliovirus particles and f_2 bacteriophages in sand. They observed that 93 percent of poliovirus were retained on the sand particles and similar results were achieved for f_2 bacteriophages also.

Carlson, et al. (1968) observed that both bacteriophages and poliovirus are adsorbed to common clays such as Kaolinite, illite and montmorillonite. Significant poliovirus adsorption to natural turbidity in water was reported to have been observed (Schaub, et al., 1974). They observed the association of poliovirus 1 and coxsackievirus B-2 with naturally occurring suspended particulate material. They concluded that cation content of a given water is very important to the adsorption process.

III. SCOPE OF THE STUDY

From a review of the literature, it is evident that studies on virus movement in groundwater or virus retention by soils were very few. However, epidemiological evidence of waterborne viral disease transmission through contaminated groundwater supplies and the presence of these viruses in wastewater and wastewater effluents which may be disposed into land indicate that such studies are quite significant from the view point of environmental health. Furthermore, the scanty information available in this area pertains to various soil types from the United States. No study has so far been undertaken to investigate virus retention or sorption by the Indian soils. However, with continued use of septic tanks and cesspools for wastewater disposal and groundwater as a major source of water supply in the rural areas of our country, it appears quite appropriate to study the effectiveness of Indian soils in removing or retaining viruses from percolating waters. The present study was initiated to investigate the sorption or retention of virus through some selected Indian soils in laboratory systems. The study was undertaken along the following lines:

a) Batch sorption tests to investigate the relative sorptive capacities of four selected Indian soils, viz.,

Beach sand from Malabar (Kerala), Black Cotton soil (Madhya Pradesh), Kanpur silt (Uttar Pradesh) and Lateritic soil (Kerala) at different pH values, and

b) column studies using these four soils to study their relative effectiveness in removing viruses from percolating waters.

IV. MATERIALS AND METHODS

A. Materials

(1) Selection of the model virus

Bacterial virus MS2 (MS2 phage) against Escherichia coli was selected as the model virus for this study because of its resemblance to human enteroviruses (polio, coxsackie, and echoviruses) in size, shape, and type of nucleic acid. Enteroviruses belong to the picornavirinae subfamily of the napoviridae family of the naked ribonucleic acid-containing viruses with cubic symmetry whereas MS2 phage belongs to the androphagovirinae subfamily of the same family (Goodheart, 1969). MS2 phage has a single stranded RNA core surrounded by a lipid-free protein coat (isoelectric point, 3.9). It is polyhedron in structure having a diameter of 25 nm, and molecular weight of 3.7×10^{-6} (Overby, et al., 1966). MS2 phage is similar to poliovirus in coat protein composition except that the amino acid residue histidine is absent.

Bacteriophages behave in the same way as enteroviruses in most of the cases (Chang, et al., 1958, and Gilcreas and Kelly, 1955). It was believed that the difference in properties and behaviour among the naturally occurring viruses of concern, i.e., enteroviruses and those between the wild and attenuated laboratory strains of such viruses would offset the

differences in behaviour between those viruses and MS2 phage. A study by the Metropolitan Water Board, London (1971-73) on virus removal by slow sand filtration showed practically identical results with poliovirus and MS2 phage. Further, bacteriophage f_2 (a variant of MS2 phage) was found to survive much longer than poliovirus in sand column saturated with distilled water or oxidation pond effluent (Lefler and Kott, 1974). Consequently data on sorption of MS2 phage or retention from percolating water by soils would be of practical significance.

(2) Soils

Four different types of soils, viz., Beach sand (Malabar, Kerala), Black Cotton soil (Madhya Pradesh), Kanpur silt (Uttar Pradesh) and Lateritic soil (Kerala) were selected for this study. The selection of soils was so made that each soil differs from the other in clay content, cation exchange capacity, organic content, etc. Also, another major factor in the selection of the soils was their occurrence in areas where percolating water may enter directly and in quantity into the groundwater body that provides the major source of water supply to domestic wells as well as community wells.

(3) Biological media

L Broth

(Constituents per liter of water)

Bacto-Tryptone (Difco)	10.0 g

Yeast Extract (Difco)	5.0 g
NaCl	10.0 g
Glucose	1.0 g
2 M $CaCl_2$	1.0 ml

pH adjusted to 7.0 with 1 N NaOH

L Agar

L Broth plus 15 - 20 g/l Agar Agar (Japan)

Soft Agar

L Broth plus 10 g/l Bacto Agar (Difco)

Phage Buffer

(Constituents per liter of water)

Tris(hydroxy methyl)aminomethane	6.06 g
NaCl	5.85 g

pH adjusted to 7.6 with 1 N HCl

(4) Water

The distilled water was obtained from the laboratory distillation plant. The groundwater used in this study was collected prior to chlorimation from the tube well no. 4 located inside the campus of the Indian Institute of Technology, Kanpur. The chemical analysis of the water is shown in Table 5.

(5) Glassware

All glassware used in this study were soaked overnight in 0.3 percent Teepol B-300 (manufactured by Surfactants

TABLE 5

Chemical Analysis of Tube Well No.4 Water

Constituents	Concentration, in milligrams per liter (except pH)
pH	7.8 - 8.0
Conductance	770 - 980 μmhos/cm
Alkalinity (HCO_3^-)	440 - 450 (as $CaCO_3$)
Hardness	200 (as $CaCO_3$)
Calcium	46 - 50 (as $CaCO_3$)
Magnesium	150 - 154 (as $CaCO_3$)
Chlorides	20 - 21
Sulphates	24 - 25
Phosphates	0.1 - 0.2
Iron	0.2 - 0.3
Manganese	0.2 - 0.3
Silica	23 - 24
Sodium + Potassium	180 - 185
Turbidity	Not detectable

Private Ltd., Bombay, India) to minimize virus sorption to glassware, followed by washing with tap water and rinsed in distilled water. Sterilization was accomplished in a hot-air oven at 180°C for 2 hr or longer.

B. Methods

(1) Preparation and enumeration of MS2 phage

The initial stock cultures of MS2 phage and its host, Escherichia coli A19, were obtained from the Environmental Engineering Laboratory, University of Illinois. Subsequent suspensions were prepared according to the following procedure.

An 1 - liter flask containing 900 ml of L Broth and equipped with an aeration device was inoculated with overnight grown Escherichia coli A19 so as to get an A_{660} of 0.06 - 0.10. The flask was maintained at 37°C and aerated until the cell culture reached an A_{660} of 0.15 - 0.20. This corresponds to an early log growth phase of Escherichia coli A19 (5×10^7 /ml). The required amount of MS2 phage stock was then added at a multiplicity of 6 - 10. The aeration of the flask at 37°C was continued with A_{660} measurements at intervals. First the absorbance would increase and later it might level off or gradually decrease indicating lysis. Five ml of chloroform was added after lysis had started and the lysate was stirred at a high speed for 1 min using a magnetic stirrer. It was then kept at 4°C and stirred every 5 min. After 30 min, the

lysate was centrifuged at low speed (5,900Xg for 10 min) to remove bacterial cell debris followed by high speed centrifugation (23000Xg for 2.5 hr) in a super speed refrigerated centrifuge (Janetzki Model K24). The virus pellet was resuspended in 5 ml of phage buffer and titered. The suspension was then stored at 4°C for future use.

Soft agar technique of Adams (1959) as followed by Chaudhuri (1969) was adopted for enumeration of MS2 phage.

Before assaying, the sample was diluted in L Broth to yield 100 - 300 plaques per plate. A liquid top-agar mixture consisting of about 3 ml of soft agar at 45°C, 0.3 ml of a log growth phase culture of Escherichia coli A19 cells, and 0.1 ml (unless otherwise specified) of the diluted virus sample was plated on solidified bottom agar (L Agar) plates and incubated at 37°C for 6 - 8 hr. Plaques were counted with the aid of a Quebeck colony counter and reported as plaque forming units per ml (PFU/ml). Triplicate plates were prepared from each sample to increase accuracy.

(2) Characterisation and preparation of soils

The physical and chemical characteristics of the selected soils used in this study are shown in Table 6.

Particle size distribution for the soil samples was done by sieve analysis and hydrometer analysis methods. Cation exchange capacities were determined using the method described

TABLE 6

Physical and Chemical Characteristics of Soils

Type of Soil	Grain size analysis percent			CEC m.eq. per 100g	Specific gravity	pH	Organic content percent	Constituent, commonly found$^{\varphi}$	Max. effluent application rate $l/m^2/d$
	Sand	Silt	Clay						
Beach sand	100	-	-	-	2.6	8.0	0.2	quartz, shell material	204
Black Cotton soil	25	46.8	28.2	8.3	2.65	7.8	1.2	montmorillonite, quartz, org. matter	37
Kanpur silt	14.88	75.12	10.0	3.2	2.58	8.2	0.9	illite, quartz, kaolinite	37
Lateritic soil	38.8	28.7	32.5	5.9	2.69	7.0	0.4	kaolinite, quartz, oxides of iron and aluminium	91

φ Gokhale, 1975

by Grim (1955). Soil pH was measured according to the method given by Peeach (1965).

Determination of soil organic matter was done using hydrogen peroxide (Gokhale, 1975). A known amount of oven dried (100 - 110°C) soil was saturated overnight with hydrogen peroxide, filtered through a Whatman No. 42 filter paper, washed several times with glass distilled water, dried in the oven at 100 - 110°C and final weight was taken. From the difference in weights, the organic matter content was determined.

Percolation rate for each soil was determined using a method similar to the one recommended for septic tank percolation system (IS: 2470 - Part I, 1968). In the procedure, the time required for a 15 cm head of water to drop 2.5 cm through a saturated column of soil was recorded and the corresponding maximum rate of effluent application for each soil was determined.

For sorption tests, the soil samples were ground in a ball mill and sieved through B.S.S. No. 200. The samples were then autoclaved, air dried and stored for future use. For column studies, soil samples as obtained were used.

(3) Experimental techniques

a) Batch sorption tests

The reaction mixture for batch sorption tests included 20 ml buffer of desired pH value (0.2 M phosphate buffer for

pH 6 and 7, and 0.2 M borate buffer for pH 8.4), 180 ml distilled water, appropriate concentration of MS2 phage and 2 g of soil. For tests at pH 7.8, 200 ml of the groundwater were used instead of buffer and distilled water. MS2 phage input concentration was maintained at 1.1×10^5 PFU/ml for all tests except for the one with Lateritic soil for which a concentration of 1.1×10^6 PFU/ml was used. Bottles containing the reaction mixture were kept on a rotary shaker and samples withdrawn at 5, 15, 30, 45, 60, 80 and 100 min, centrifuged at 5900Xg for 10 min and the supernatant assayed for MS2 phage. A control was also run to account for any virus inactivation during the experiment. For equilibria studies (pH 7.0), the virus concentration was maintained constant and the concentration of soil was varied.

(b) Column studies

Cylindrical glass columns, 60 x 4.6 cm, provided with a perforated glass plate at the bottom were used for the column studies. Soil in the column was supported by placing above the perforated glass plate about 5 cm glasswool treated with Teepol B-300 to minimize virus sorption. These columns were filled with soil samples upto the desired thickness (15 cm for Black Cotton soil and Kanpur silt, 7.5 cm for Lateritic soil, and 15,30, and 37.5 cm for Beach sand) and conditioned by saturating them several times with distilled water prior to experimentation.

Tube well No. 4 water was used for preparing the virus feed solution which ranged from 1.05×10^6 PFU/ml for Beach sand, Black Cotton soil and Kanpur silt, and 2.10×10^6 PFU/ml for Lateritic soil. The total volume of the feed solution required for a particular soil column per day was computed using the percolation test data and the column was dosed intermittently four times a day at an interval of 2 hr for Beach sand, Black Cotton soil and Lateritic soil, and three times a day at an interval of 3 hr for Kanpur silt. The feed solution was carefully poured into the column in such a manner that it did not pond up more than one cm. For Kanpur silt, an application rate equal to the one obtained for Black Cotton soil was used as it showed extremely slow percolating characteristics. The intermittent feeding was continued till sufficient virus breakthrough was observed for a particular soil column. A control column without soil was always employed to account for virus inactivation or loss during the experiment.

V. RESULTS AND DISCUSSION

A. Batch sorption tests

Batch sorption tests were conducted to investigate the relative sorptive capacities of the four selected soils. For virus enumeration, samples were plated in triplicate and the average values were used in computing the percent sorption.

Figure 1 - 4 show the kinetics of sorption of MS2 phage on Beach sand, Black Cotton soil, Kanpur silt and Lateritic soil, respectively at pH values of 6.0, 7.0, and 8.4. Tube well No. 4 water (pH 7.8; ionic strength 0.02) was also used to study virus sorption by the selected soils in a natural groundwater environment. The results show that bulk of the sorption takes place during the first 30-40 min and a plateau is reached in about 100 min. For all the soils, both the rate of sorption and the sorptive capacity decrease with increase in pH; however, with the natural groundwater both the rate of sorption and sorptive capacity are observed to be the maximum. Among the four selected soils, Lateritic soil is the most efficient in sorbing the model virus. The maximum rate of virus sorption (Tube well No. 4 water at pH 7.8) for the four soils are as follows: Lateritic soil - 2.16×10^7 PFU/g/min; Black Cotton soil 1.87×10^6 PFU/g/min; Kanpur silt - 1.71×10^6 PFU/g/min; and Beach sand - 1.69×10^6 PFU/g/min.

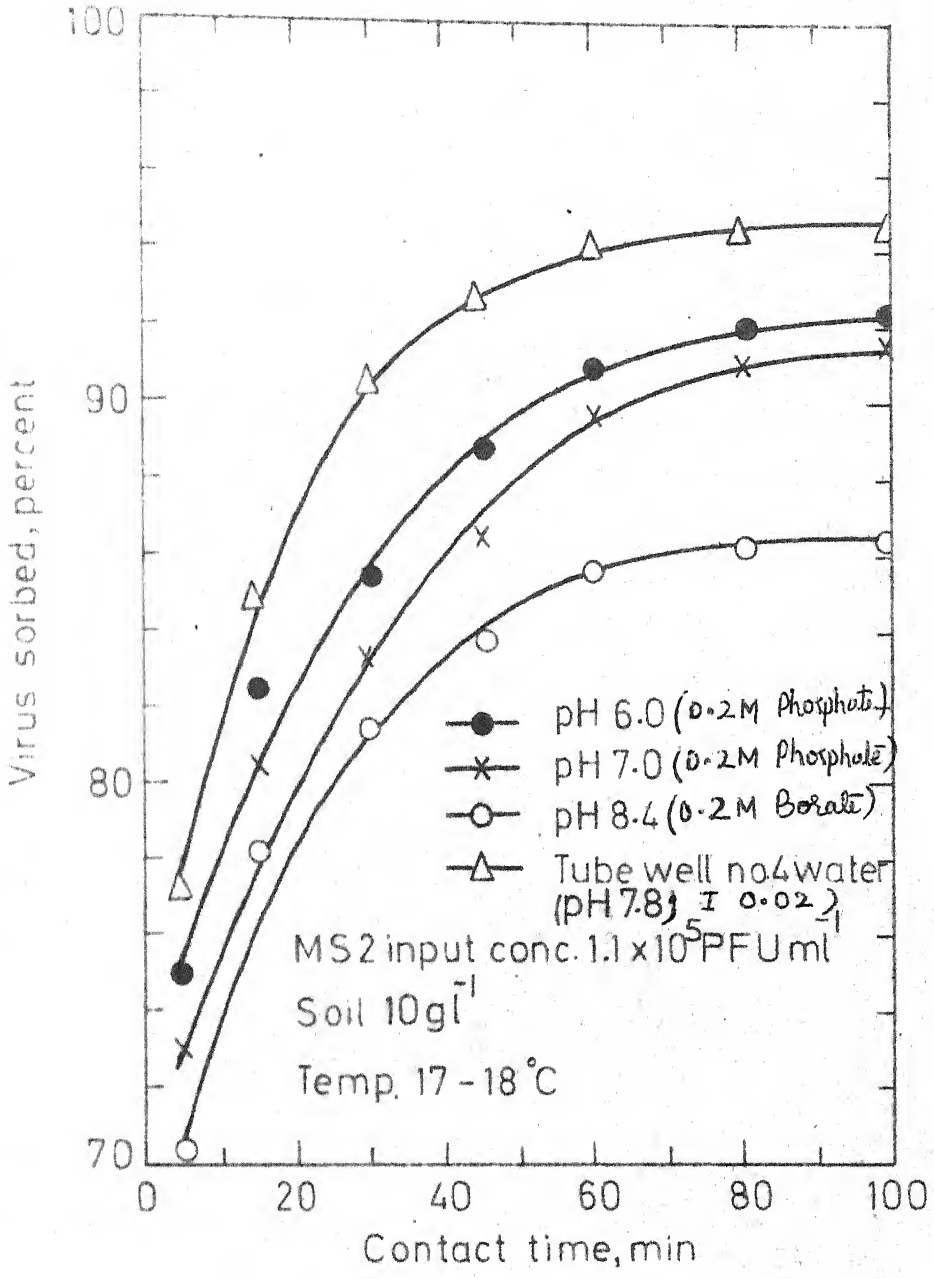

Fig. 1 Kinetics of sorption of MS2 phage on Beach sand

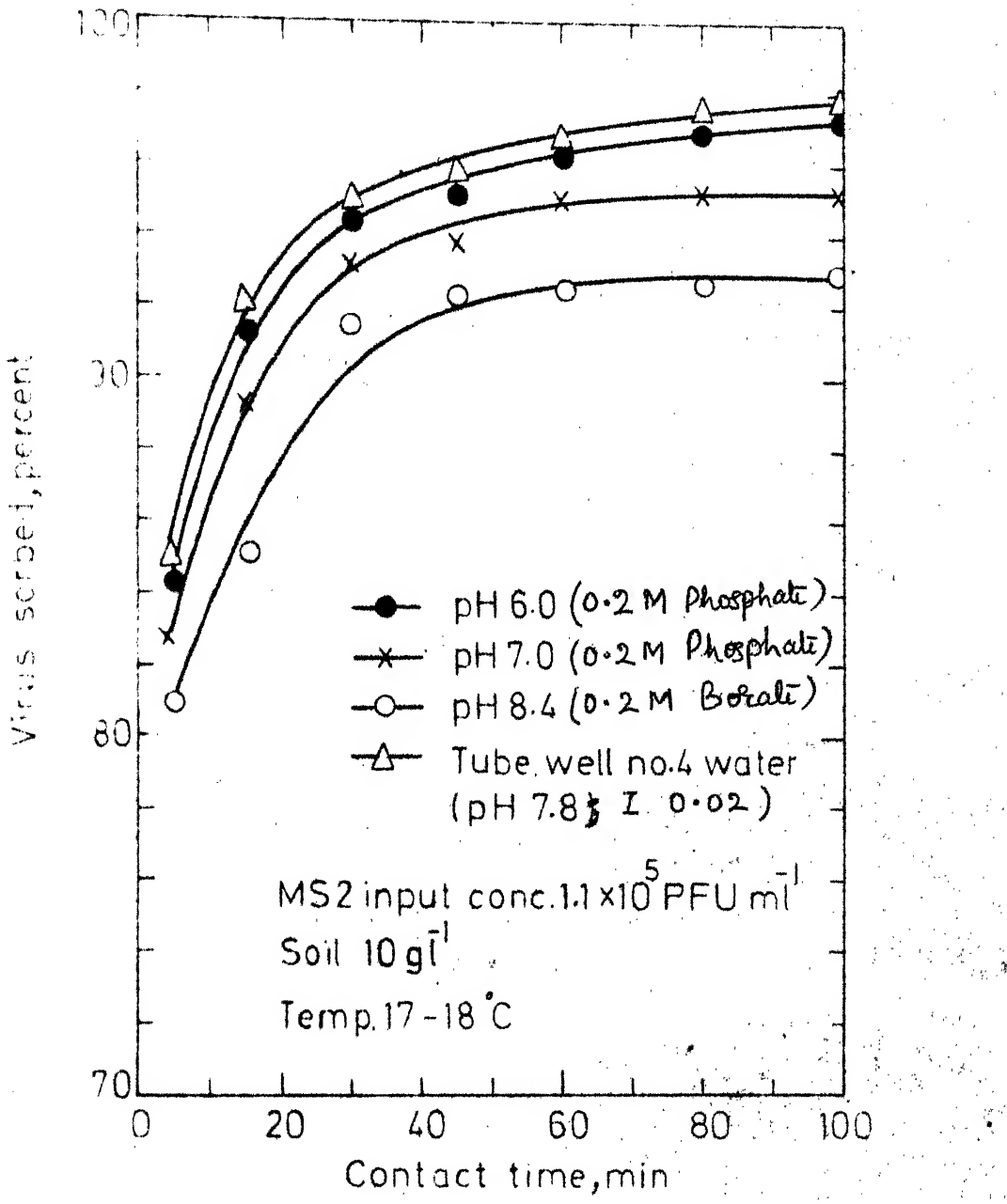

Fig. 2 Kinetics of sorption of MS2 phage on Black cotton soil

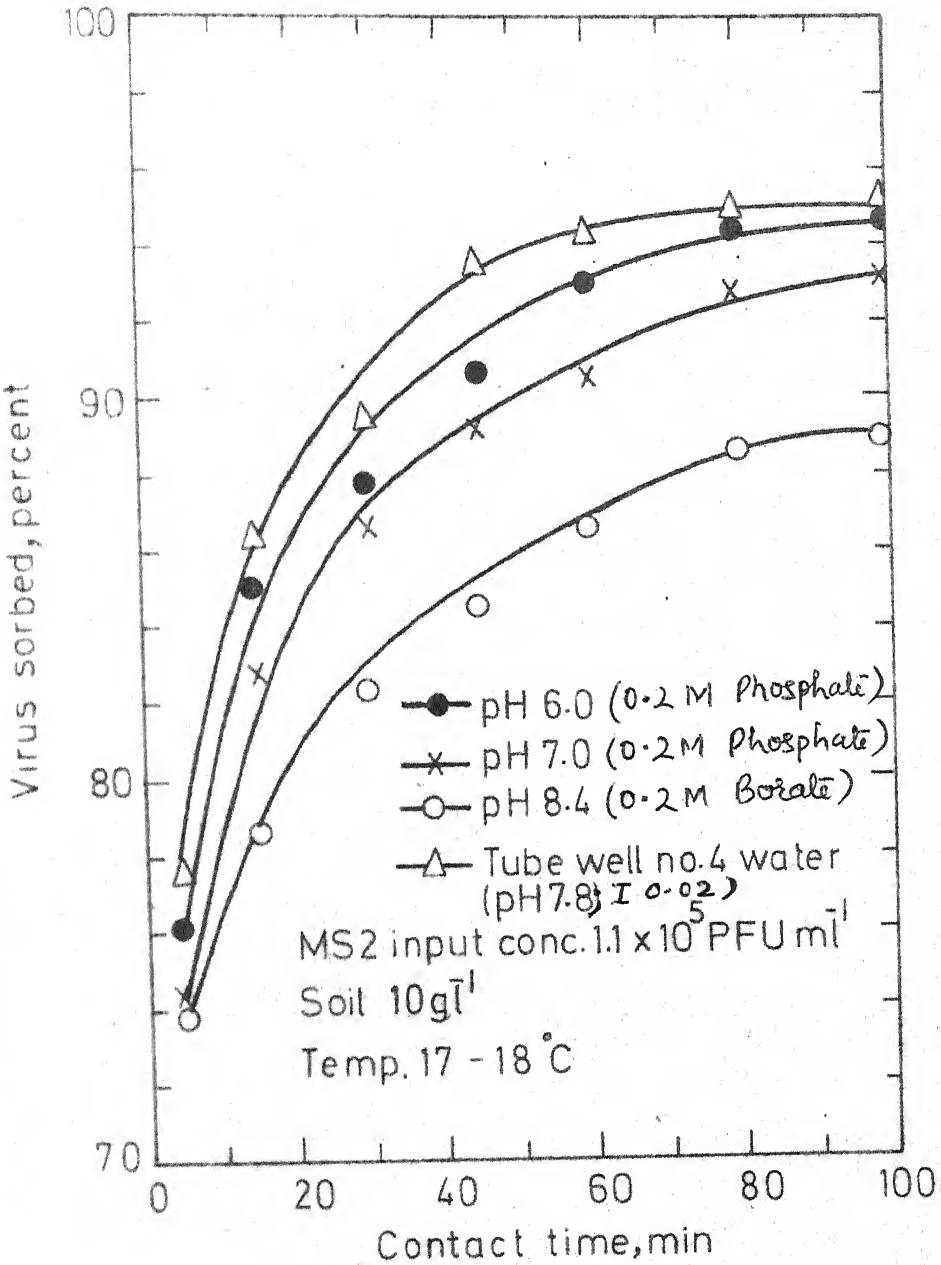

Fig. 3 Kinetics of sorption of MS2 phage on Kanpur silt

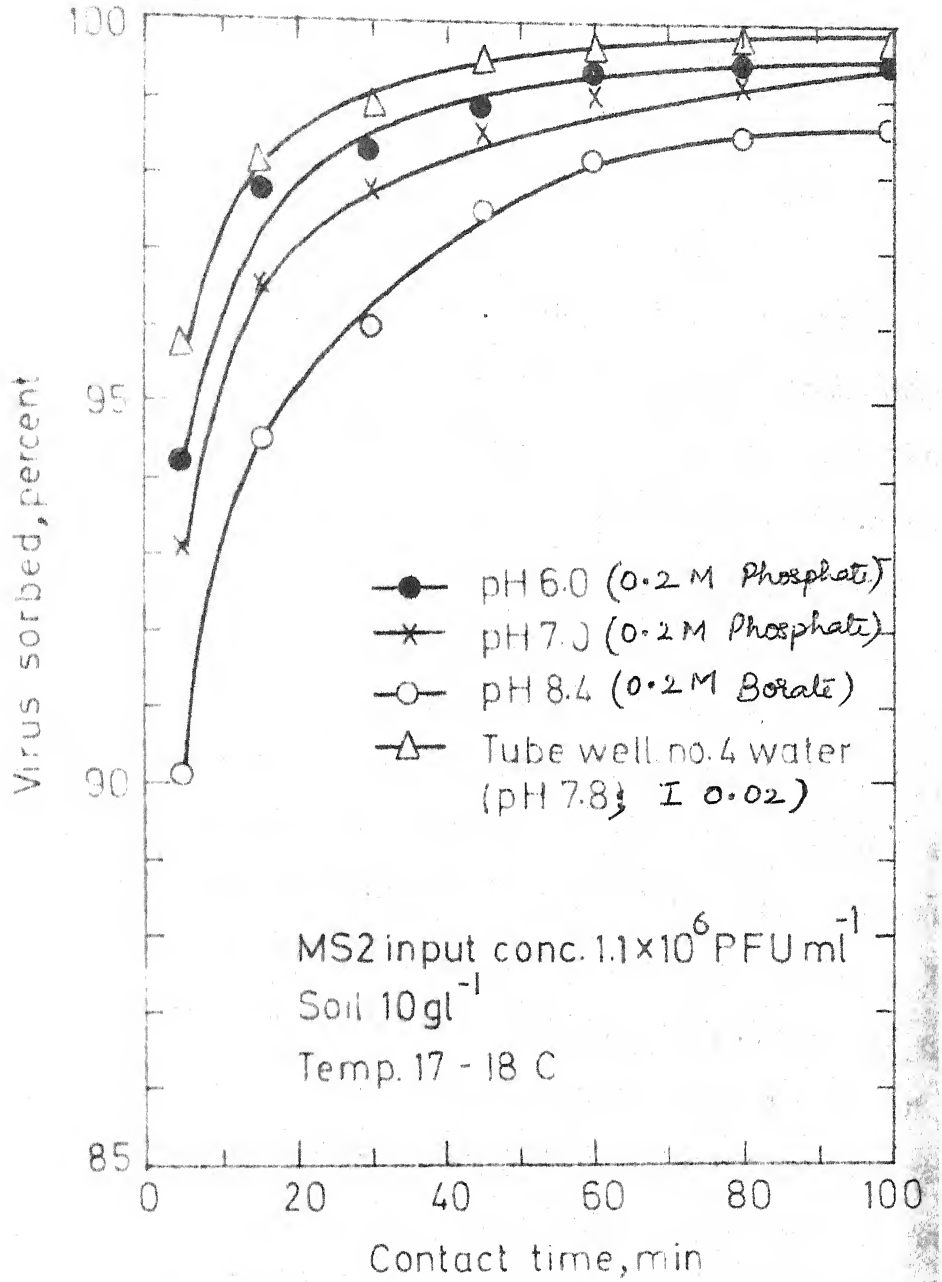

Fig. 4 Kinetics of sorption of MS2 phage on Lateritic soil

In general, virus sorption decreases with increase in pH (Fig. 5). This trend is presumably due to increased negativity of soil (Bear, 1964) as well as the virus (Overby, et al., 1966) with increase in pH. With increase in pH a decrease in the ionization of the amino groups and an increase in the ionization of the carboxyl groups of viral protein would occur rendering the virus particles more electronegative. At the same time, the negative charge on the soil particles increases. Consequently repulsion would increase resulting in a decrease in sorption. With tube well No.4 water (pH 7.8; I 0.02) virus sorption were 94.6, 97.6, 95.0 and 99.7 per cent for Beach sand, Black Cotton soil, Kanpur silt and Lateritic soil respectively. Relatively high sorption, both in terms of rate of sorption and sorptive capacity, observed with the virus-soil-groundwater system may be attributed to the presence of polyvalent cations leading to enhanced virus-cation-soil bridging (Carlson, et al., 1968).

Figure 6 shows the adsorption isotherm for the selected soils according to the Freundlich adsorption equation ($X/M = KC_e^n$ where, X = virus sorbed in PFU/ml; M = weight of soil in g; C_e = virus remaining at equilibrium, PFU/ml/g; K, n = constants). In these experiments a 2 hr contact time was used since it was observed from the kinetic studies that sorption reached a pleateau after 100 min. The sorptive capacities determined for the four soils are also shown in Fig.6. Eliassen et al. (1964) and Eliassen, et al. (1967) also observed conformity of their sorption test data to the Freundlich equation. The

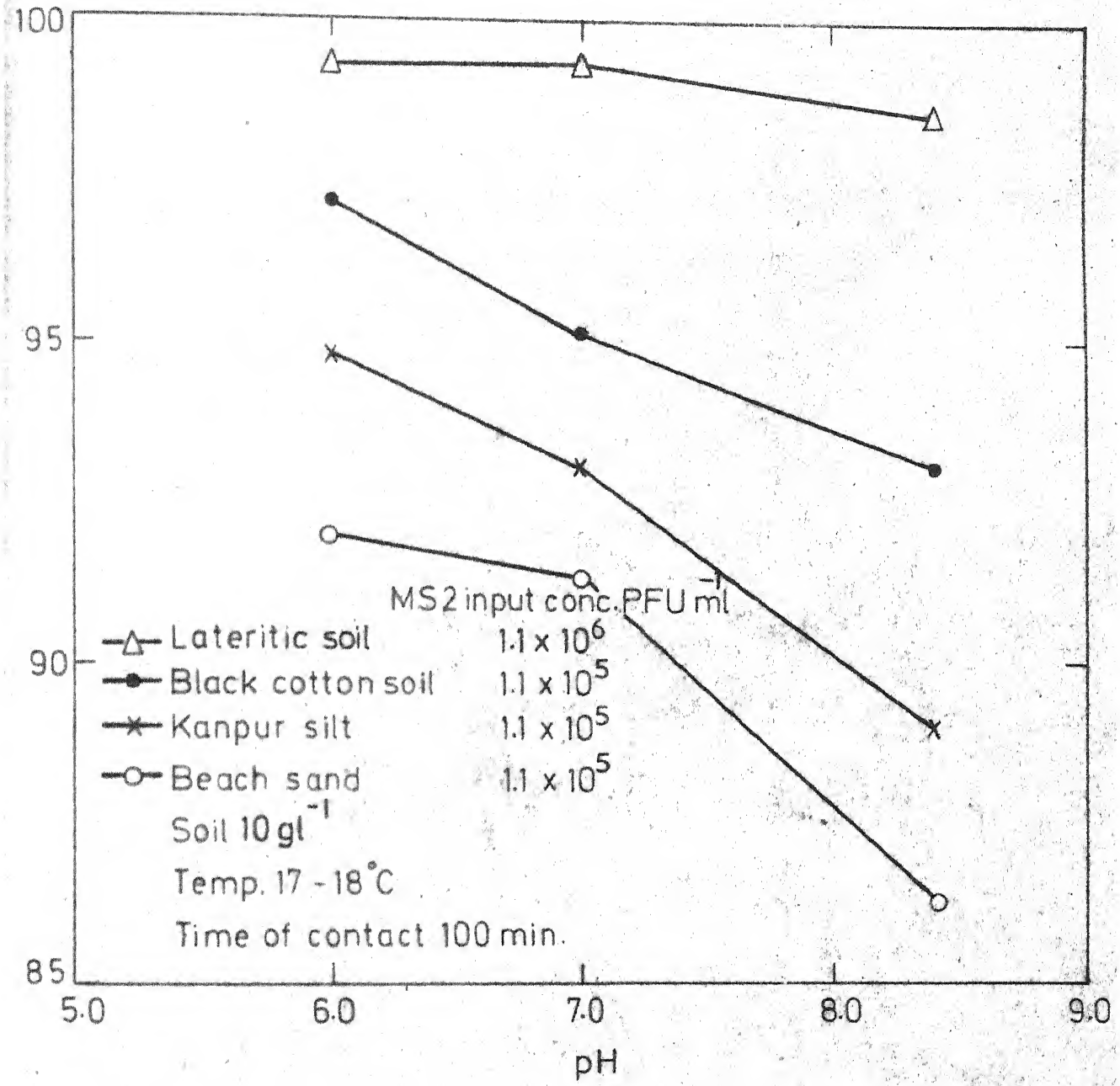

Fig. 5 Effect of pH on sorption of MS2 phage by various soils

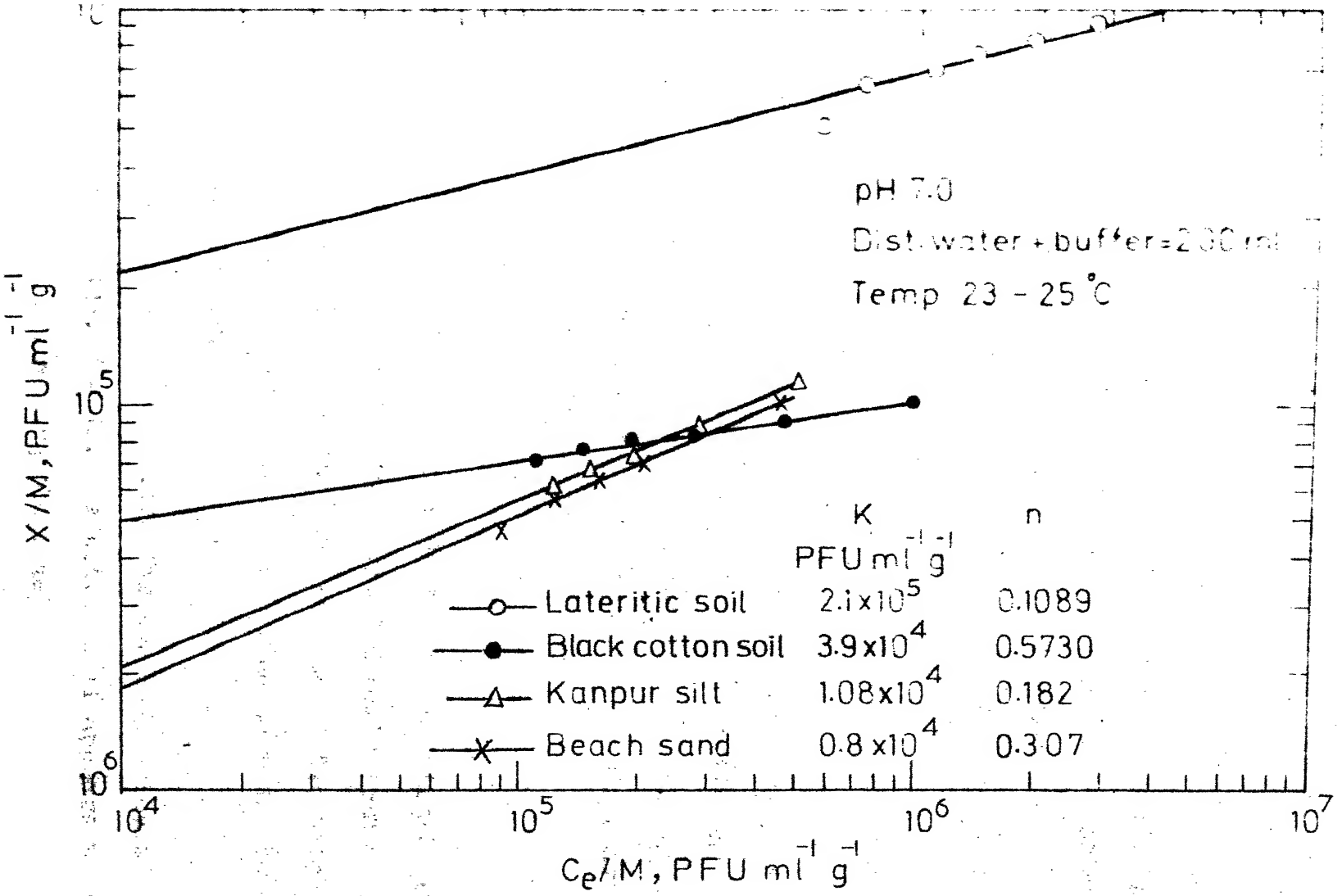

Fig.6 Freundlich sorption isotherm for MS2 phage and various soils

difference in sorptive capacities of the four selected soils may be explained in terms of their clay content. The soil with the highest clay content, viz., Lateritic soil has the maximum virus sorptive capacity. No correlation between virus sorption and the cation exchange capacity of soil is apparent. Filmer and Corey (1966) observed that soils with higher clay content were more efficient in removing virus-sized albumin particles. Carlson, et al. (1968) also observed that common clays such as kaolinite, illite and montmorillonite were very efficient in sorbing viruses from water. They also reported that the sorptive capacity of kaolinite was higher than that of illite and montmorillonite. Schaub, et al. (1974) also observed that viruses were sorbed well onto clays constituting turbidity in natural waters.

B. Column studies

Column studies were conducted to investigate the removal of the model virus by the four selected soils from percolating waters. It was believed that the results of the batch sorption tests coupled with those from column studies would be of practical significance.

The breakthrough curves for the four selected soils are shown in Figs. 7 - 10. Lateritic soil appears to be the most effective in removing the model virus from percolating water. Rapid breakthrough of MS2 phage through the Beach

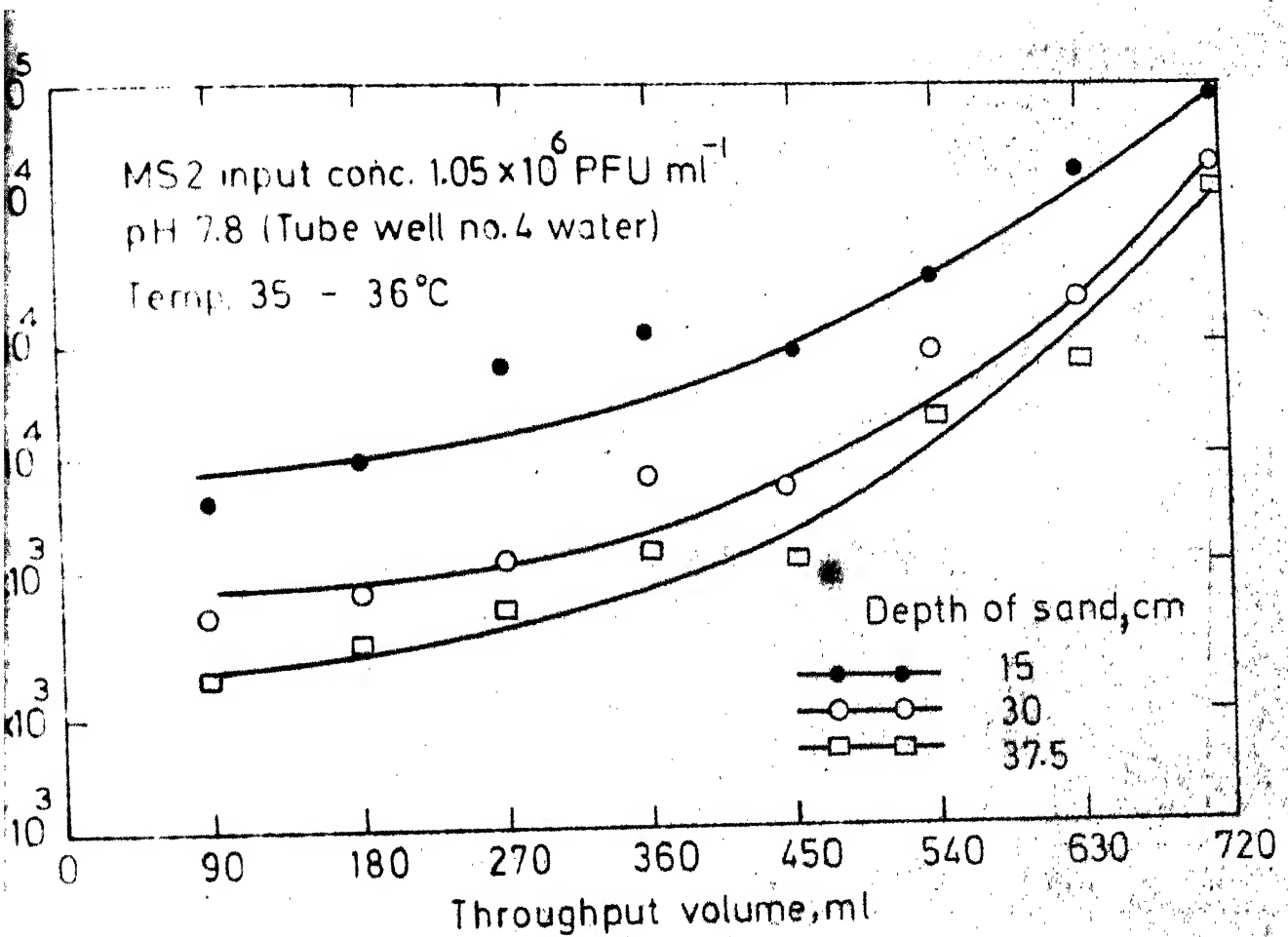

Fig. 7 Virus breakthrough curve for Beach sc

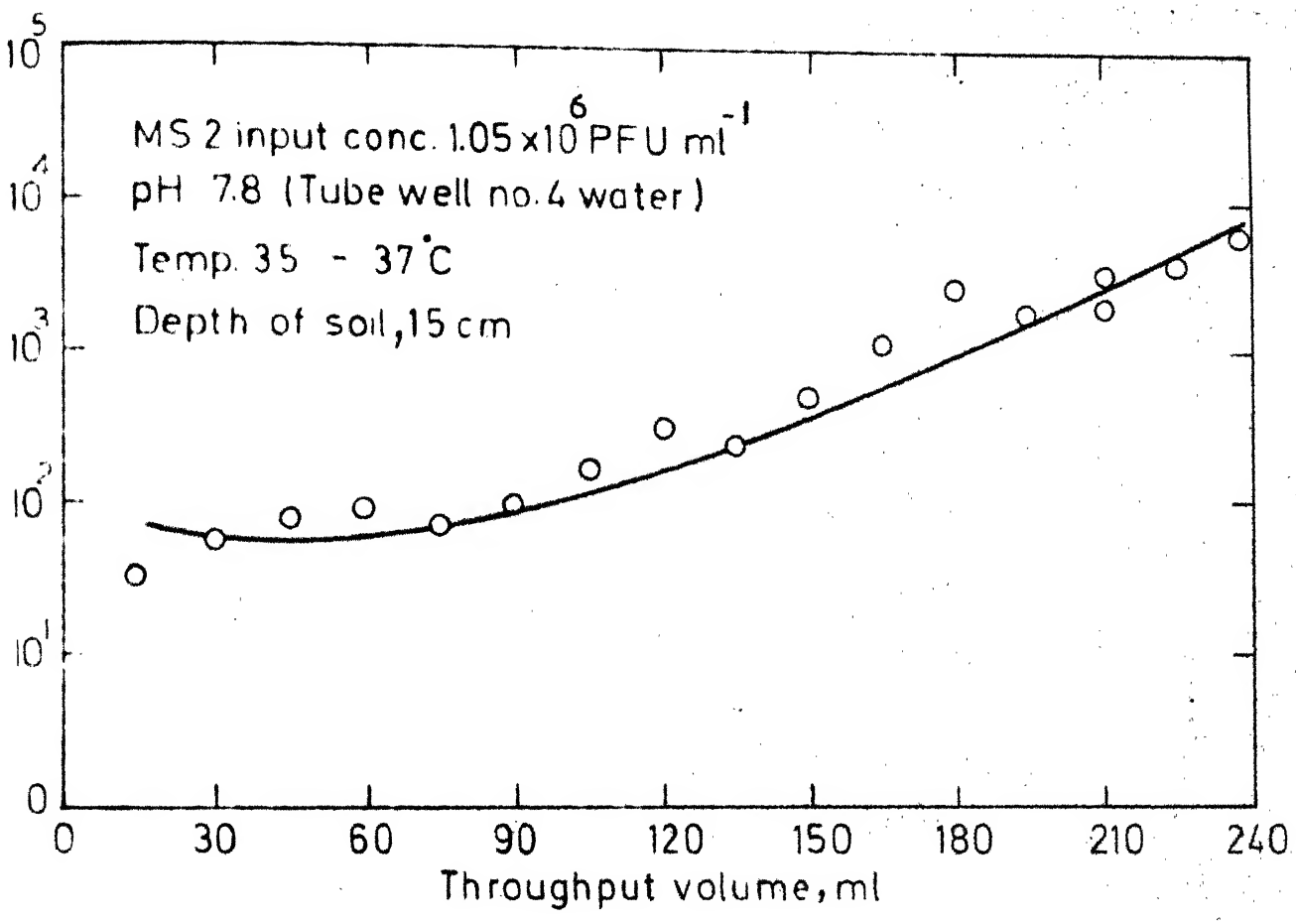

Fig. 8 Virus breakthrough curve for Black cotton

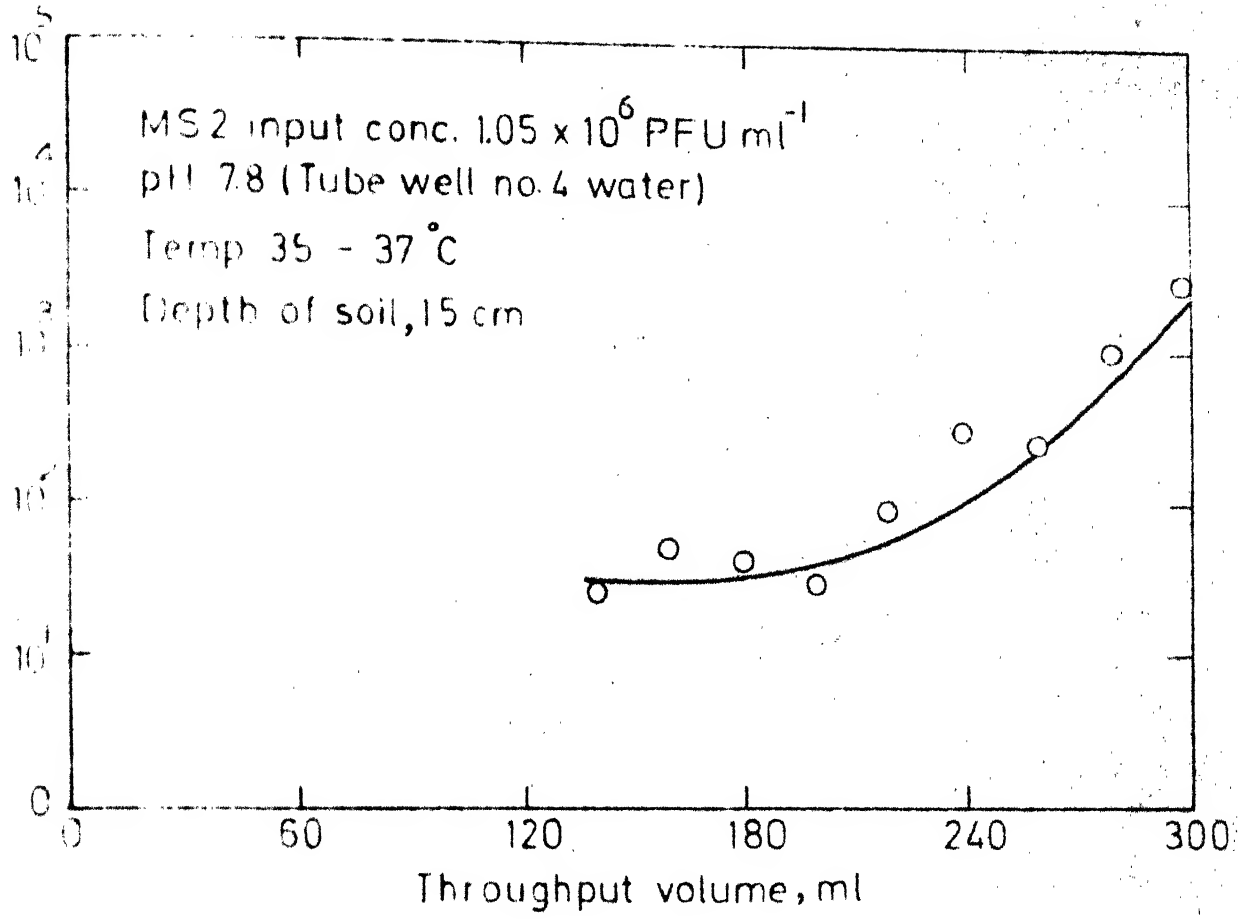

Fig 9 Virus breakthrough curve for Kanpur silt

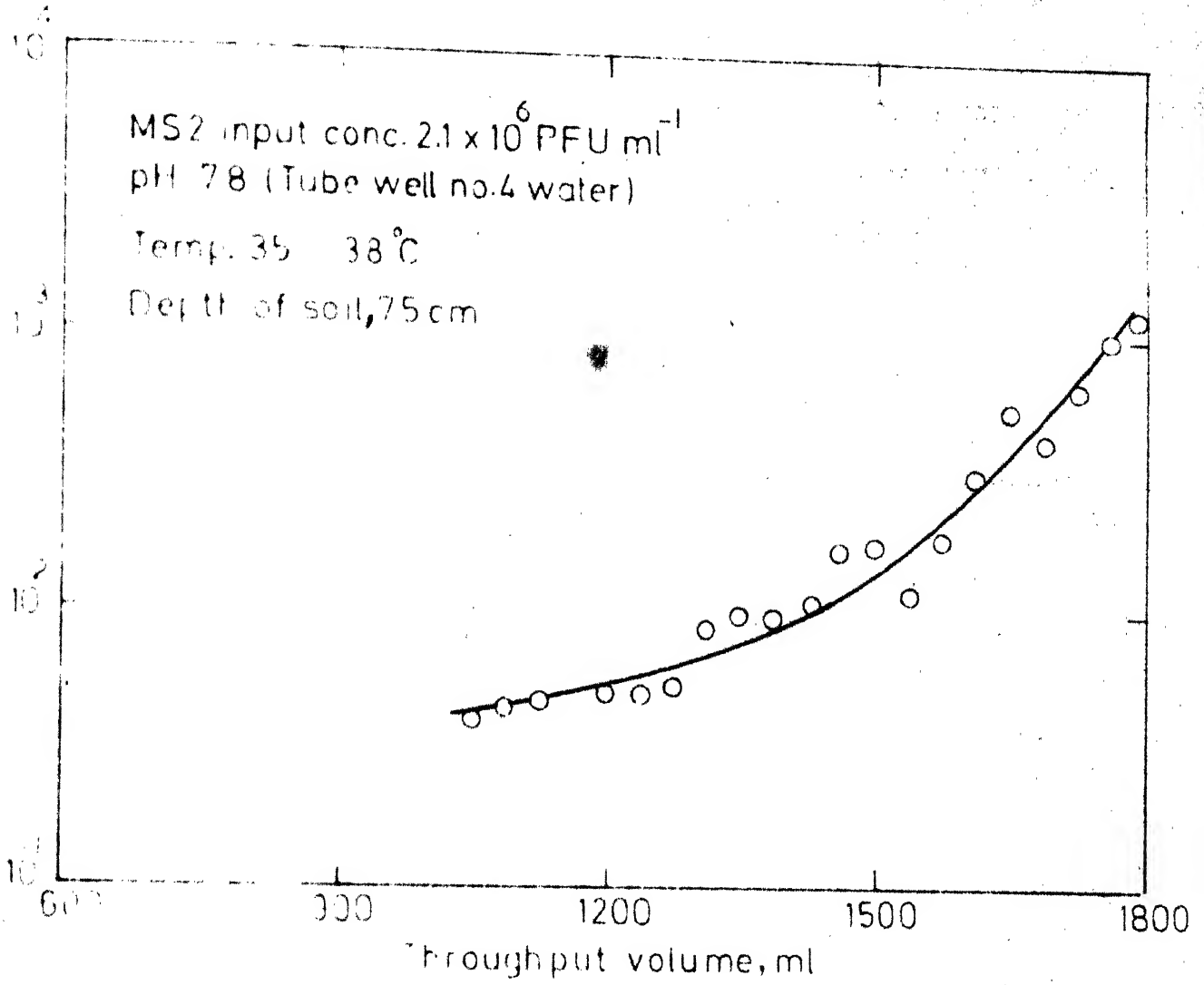

Fig 10 Virus breakthrough curve for Lateritic soil

sand columns may be explained by rapid drainage through the column and the absence of clay. Even though the clay content of Black Cotton soil was more than that of Kanpur silt, the breakthrough of MS2 phage through the Black Cotton soil column of same depth occurred faster. The higher removal of virus by Kanpur silt column might be due to the slow drainage of percolant through the column.

Few spot tests were conducted to study the fate of the virus particles retained in the soil column. It was observed that the majority of the retained viruses remained active following the sorption on soil.

VI. SUMMARY AND CONCLUSIONS

The results of the present study show that the selected Indian soils, viz., Lateritic soil, Black Cotton soil and Kanpur silt are effective in removal of viruses from water both in terms of batch sorption tests as well as the column studies. Malabar Beach sand is comparatively poor for removing viruses from water. The sorptive capacity of soil was found to be dependent on its clay content and the pH of the system. No apparent correlation was observed between the sorption of viruses and cation exchange capacities of soils. From the equilibrium sorption tests, the sorptive capacities of the soils were as follows: Beach sand- 1.6×10^6 PFU/g; Black Cotton soil - 7.8×10^6 PFU/g; Kanpur silt - 2.16×10^6 PFU/g; and Lateritic soil - 4.2×10^7 PFU/g. From the column studies it was observed that a 7.5 cm depth of Lateritic soil was much more efficient than Black Cotton soil and Kanpur silt of 15 cm depth in removing the model viruses. Breakthrough of the model virus through sand was immediate apparently due to rapid drainage of percolant through the sand columns and its poor sorption potential.

Based on the findings of this investigation using a model bacterial virus, the following conclusions may be drawn:

1) The selected soils except Malabar Beach sand, were effective in removing viruses from water both in terms of batch sorption tests and column studies.

2) Virus sorption by soil follows Freundlich sorption isotherm, and is dependent on the clay content of the soil and pH of the system. Presence of polyvalent cations enhances virus sorption.

3) Virus particles retained in the soil are not inactivated following sorption.

VII. ENGINEERING SIGNIFICANCE AND SUGGESTIONS FOR FUTURE WORK

A. Engineering significance

The present study is very significant from the viewpoint of environmental engineering as this is the first study undertaken to investigate the removal of viruses by Indian soils. In majority of our urban and suburban areas the most predominant method of human waste disposal is through septic tanks and cesspools. In our rural areas the practice of defecating on land is still in vogue. Consequently, removal of viruses by soil from percolating water becomes extremely important. Furthermore, it is felt that this study will provide enough impetus and background information for future laboratory as well as field studies in this area designed to assess the potential problem of groundwater pollution due to land disposal of virus-laden effluents.

B. Suggestions for future work

Based on the results of this study it is felt that further work should be pursued in the following areas:

1) More studies (both under intermittent as well as saturated flow conditions) should be undertaken to evaluate soil sample from different parts of the country in terms of

their virus retention potential. Effects of system pH, flow rate, virus concentration etc., on virus retention or movement through soil should be investigated. Also, the fate of the viruses retained on the soil needs to be evaluated.

2) Future laboratory and field studies in this area should employ enterovirus(es) with input concentrations comparable to that normally encountered in percolating waters. This would necessitate the use of a suitable virus concentration technique.

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APPENDIX

TABLE A1

Equilibrium Sorptive Capacities of Beach Sand

Weight of soil, G (M)	Virus input conc. x 10^5 PFU/ml.	Viruses remaining x 10^4 PFU/ml. (C_e)	Viruses sorbed x 10^4 PFU/ml. (X)	X/M x 10^4 PFU/ml/G	C_e/M x 10^5 PFU/ml/G
0.1	1.1	9.8	1.2	12.0	9.8
0.2	1.1	9.0	2.0	10.0	4.5
0.3	1.1	8.5	2.5	8.33	2.83
0.4	1.1	8.2	2.8	7.0	2.05
0.5	1.1	7.8	3.2	6.4	1.56
0.6	1.1	7.5	3.5	5.83	1.25
0.8	1.1	7.2	3.8	4.75	0.90
1.0	1.1	6.0	5.0	5.0	0.60
2.0	1.1	0.0	11.0	5.5	-

pH 7.0, Temp. 23-25°C, Distilled water + Buffer = 200 ml.

TABLE 4.2

Equilibrium Sorptive Capacities of Black Cotton Soil

Weight of soil, G (M)	Virus input conc. x 10^5 PFU/ml.	Viruses remaining x10^4 PFU/ml. (C_e)	Viruses sorbed x10^4 PFU/ml. (X)	X/M x 10^4 PFU/ml/g	C_e/M x 10^5 PFU/ml/G
0.1	1.1	10.0	1.0	10.0	10.0
0.2	1.1	9.2	1.8	9.0	4.6
0.3	1.1	8.5	2.5	8.33	2.83
0.4	1.1	7.8	3.2	8.0	1.95
0.5	1.1	7.2	3.8	7.6	1.44
0.6	1.1	6.7	4.3	7.16	1.11
0.8	1.1	5.88	5.12	6.4	0.735
1.0	1.1	5.5	5.5	5.5	0.55
2.0	1.1	0	11.0	5.5	-

pH 7.0, Temp. 23 - 25°C, Distilled Water + Buffer = 200 ml.

TABLE A3

Equilibrium Sorptive Capacities of Rampur Silt

Weight of soil, S (M)	Virus input conc. $\times 10^5$ IFU/ml.	Viruses remaining $\times 10^4$ IFU/ml. (C_e)	Viruses sorbed $\times 10^4$ IFU/ml. (X)	X/M $\times 10^4$ IFU/ml/g	C_e/M $\times 10^5$ IFU/ml/g
0.1	1.1	9.9	1.1	11.0	9.0
0.2	1.1	8.9	2.1	10.5	4.95
0.3	1.1	8.4	2.6	8.66	2.80
0.4	1.1	8.0	3.0	7.50	2.0
0.5	1.1	7.7	3.3	6.6	1.54
0.6	1.1	7.4	3.6	6.0	1.23
0.8	1.1	6.2	4.8	6.0	0.75
1.0	1.1	5.5	5.5	5.5	0.55
2.0	1.1	0.0	11.0	5.5	-

pH 7.0, Temp. 23-25°C, Distilled Water + Buffer = 200 ml.

TABLE 4.2

Equilibrium Sorptive Capacities of Lateritic Soils

Weight of soil, g (M)	Virus input conc. x 10^6 PFU/ml.	Viruses remaining x 10^5 PFU/ml (C_e)	Viruses sorted x 10^5 PFU/ml (X)	X/M x 10^6 PFU/ml/g	C_e/M x 10^6 PFU/ml/g
0.1	1.1	9.78	1.22	1.22	9.78
0.2	1.1	9.0	2.0	1.0	4.50
0.3	1.1	8.28	2.72	0.90	2.76
0.4	1.1	7.78	3.22	0.80	1.94
0.5	1.1	7.15	3.85	0.77	1.43
0.6	1.1	6.77	4.23	0.71	1.13
0.8	1.1	5.88	5.12	0.64	0.74
1.0	1.1	5.10	5.90	0.59	0.51
2.0	1.1	0.0	11.0	0.55	-

pH 7.0, Temp. 23-25^{o}C, Distilled Water + Buffer = 200 ml.

TABLE 45

Average Breakthrough Numbers (PFU/ml) of MS2 Phage for Beach Sand

Sampling day	Sampling runs	Average breakthrough numbers, PFU/ml 15 cm. soil column	30 cm. soil column	37.5 cm. soil column
First Day	1	7.9×10^3	3.98×10^3	2.63×10^3
	2	1.02×10^4	4.4×10^3	3.16×10^3
	3	1.77×10^4	5.6×10^3	4.16×10^3
	4	2.23×10^4	9.7×10^3	6.3×10^3
Second Day	1	1.99×10^4	7.9×10^3	5.6×10^3
	2	3.16×10^4	1.99×10^4	1.41×10^4
	3	6.3×10^4	4.4×10^4	3.2×10^4
	4	7.9×10^4	6.3×10^4	5.6×10^4

Virus input conc. 1.05×10^6 PFU/ml, Influent application rate 204 $l/m^2/d$, Temp. 35° - 36°C, Adsorption by control column 3.8×10^2 PFU/ml.

TABLE A6

Average Breakthrough Numbers (PFU/ml) of MS2 Phage for Black Cotton Soil and Kanpur Silt.

Sampling day	Sampling runs	Average breakthrough numbers, PFU/ml 15 cm. soil column of Black Cotton	Sampling day	Sampling runs	15 cm. soil column of Kanpur silt
First Day	1	35	First Day	1	0
	2	56		2	0
	3	63		3	0
	4	70	Second Day	1	0
Second Day	1	60		2	0
	2	100		3	0
	3	177	Third Day	1	31
	4	316		2	52
Third Day	1	281		3	45
	2	562	Fourth Day	1	32
	3	1580		2	100
	4	2630		3	316
Fourth Day	1	1990	Fifth Day	1	251
	2	3090		2	1120
	3	4260		3	2500
	4	6300			

Virus input concentration 1.05×10^6 PFU/ml, Influent application rate 37 $l/m^2/d$, Temp. $35^\circ C - 37^\circ C$, Virus adsorption by control column 2.2×10^2 PFU/ml.

TABLE 47

Average Breakthrough Numbers (PFU/ml) of MS2 Phage for Lateritic Soil (15 cm. depth)

Sampling day	Sampling runs	MS2 phage PFU/ml	Sampling day	Sampling runs	MS2 phage PFU/ml.
First Day	1	0	Seventh Day	1	0
	2	0		2	0
	3	0		3	0
	4	0		4	0
Second Day	1	0	Eighth Day	1	39
	2	0		2	44
	3	0		3	50
	4	0		4	54
Third Day	1	0	Nineth Day	1	47
	2	0		2	51
	3	0		3	89
	4	0		4	100
Fourth Day	1	0	Tenth Day	1	85
	2	0		2	112
	3	0		3	158
	4	0		4	177
Fifth Day	1	0	Eleventh Day	1	125
	2	0		2	170
	3	0		3	316
	4	0		4	562
Sixth Day	1	0	Twelveth Day	1	390
	2	0		2	725
	3	0		3	960
	4	0		4	1250

Virus input concentration 2.1×10^6 PFU/ml., Influent application rate 91 $l/m^2/d$, Temp. $35^\circ - 38^\circ C$, Virus adsorptic by control column 3.31×10^2 PFU/ml.